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Research Article

Tales of the unexpected II: two new angiocarpic representatives of Russulaceae (Russulales, Basidiomycota) from tropical Southeast Asia

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Abstract

The evolution of angiocarpic fruiting bodies has occurred numerous times across Russulaceae (Russulales, Basidiomycota). A unique example of a tropical region with an unusually large number of such sequestrate taxa is the humid rainforests of Sri Lanka. In this paper, we describe two new sequestrate species of *Russula* subg. *Russula* from Sri Lanka, *Russula botrytigustata* **sp. nov.** and *Russula ciceriformis* **sp. nov.**, based on molecular phylogenetic analyses and extensive morphological descriptions. A key to all sequestrate Russulaceae known from South and Southeast Asia, including these newly described taxa, is provided. Lastly, the possible adaptive advantages that may explain the high abundance of sequestrate fungi in these rainforests are discussed.

Key words: *Cystangium*, *Gymnomyces*, hypogeous fungi, *Macowanites*, rainforest, *Russula*, sequestrate fungi, taxonomy

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Introduction

Angiocarpic fruiting bodies have evolved numerous times in different lineages of the mushroom-forming fungi (Agaricomycetes, Basidiomycota). Especially the sequestrate morphology, representing a rapid evolutionary shift from a gymnocarpic to an angiocarpic habit, has arisen many times independently (Wilson et al. 2011; Sánchez-García et al. 2020; Kuhar et al. 2023). Sequestrate species display a wide range of morphologies, often closely resembling agaricoid sister species. They may be epigeous or (semi-)hypogeous, may or may not bear a (reduced) stipe or columella, may have a (sub)lamellate to loculate hymenophore, and may produce heterotropic (ballistosporic) or orthotropic (statismosporic) spores.

An example of a family that displays a high number of transitions to a sequestrate morphology is Russulaceae (Russulales, Agaricomycetes). Although the close evolutionary relationship of these sequestrate representatives to the agaricoid members of Russulaceae was recognized very early on, their systematic history has been complicated (Malençon 1931). Older studies classified them within

different genera based on the presence of latex, occurrence of sphaerocytes in the hymenophoral trama, spore symmetry, and the presence of a stipe or columella (Kalchbrenner 1876, 1882; Cavara 1897, 1900; Mattirolo 1900; Heim 1937, 1959; Singer and Smith 1960; Kreisel 1969; Malençon 1975; Oberwinkler 1977). Since the advent of molecular techniques in mycological systematics, it has become evident that sequestrate Russulaceae are dispersed throughout the family. As a result, the angiocarpic genera *Gymnomyces*, *Martellia*, *Cystangium*, and *Elasmomyces* were synonymized with *Russula*. In contrast, most species of the sequestrate, latex-exuding genera *Gastrolactarius*, *Arcangeliella*, and *Zelleromyces* are now included in *Lactarius* (Miller et al. 2001, 2006; Nuytinck et al. 2003; Eberhardt and Verbeken 2004; Lebel and Tonkin 2007; Elliott and Trappe 2018). Three independent shifts to a sequestrate habit have also been described in *Lactifluus* (Lebel et al. 2021). To date, over 200 species of sequestrate Russulaceae have been described, with the greatest diversity within the genus *Russula*.

The diversity of sequestrate Russulaceae is best documented from temperate to arid regions in Australia and New Zealand (Bougher 1997; Lebel 1998, 2002, 2003; Bougher and Lebel 2001; Lebel and Castellano 2002), North America (Zeller and Dodge 1919; Dodge and Zeller 1936; Singer and Smith 1960; Smith 1963; Thiers 1984; Desjardin 2003; Smith et al. 2006), Southern Europe (Tulasne and Tulasne 1843; Berkeley 1844; Boudier and Patouillard 1888; Cavara 1897, 1900; Mattirolo 1900; Patouillard 1910; Soehner 1924, 1941; Zeller and Dodge 1935; Llistosella and Vidal 1995; Moreno-Arroyo et al. 1998, 2002; Calonge and Vidal 1999, 2001; Calonge and Martín 2000; Nuytinck et al. 2003; Vidal et al. 2019) and even South America (Singer and Smith 1960; Horak 1994; Trierveiler-Pereira et al. 2015). Especially in Australia and New Zealand, they seem relatively abundant (Bougher and Lebel 2001).

Their distribution in tropical climates, however, is not as well studied or understood. Tropical Africa, for instance, is unexpectedly species-poor in sequestrate Russulaceae compared with its agaricoid diversity, with only four angiocarpic taxa recorded so far: *Lactarius dolichocaulis* (Pegler) Verbeken & U. Eberh., *L. angiocarpus* Verbeken & U. Eberh., *Russula capitis-orae* (Dring) T. Lebel, and *L. megalopterus* Beenken & Sainge (Dring and Pegler 1978; Eberhardt and Verbeken 2004; Verbeken and Walleyn 2012; Beenken et al. 2016). Moreover, these taxa are known from very few collections, often from single localities, underscoring the apparent rarity of sequestrate Russulaceae on the continent. In other tropical habitats, however, the number of sequestrate species can exceed the agaricoid diversity. In tropical Southeast Asia, for example, nine taxa of sequestrate Russulaceae are known (Corner and Hawker 1953; Heim 1959; Verbeken et al. 2014). In contrast, another nine species have been described from China and Taiwan (Zhang and Yu 1990; Sang et al. 2016; Li et al. 2018; Neng and Yang 2023; Fu et al. 2025). Interestingly, five of the sequestrate *Lactarius* species described by Verbeken et al. (2014) were encountered in the same rainforest habitat in Sri Lanka: *L. pomiolens* Verbeken & Stubbe, *L. saturnisporus* Verbeken & Stubbe, *L. shoreae* Stubbe & Verbeken, *L. echinellus* Verbeken & Stubbe, and *L. echinus* Stubbe & Verbeken. This area around the Sinharaja reserve in the Sabaragamuwa Province thus seems to represent a unique biodiversity hotspot for truffle-like fungi.

In this study, we aim to build upon this exceptionally high diversity of sequestrate Russulaceae in this tropical rainforest habitat in Sri Lanka by studying collections

of non-latex-exuding sequestrate Russulaceae encountered around the Sinharaja reserve. This area is dominated by ectomycorrhizal (ECM) trees of the genera *Shorea* (Dipterocarpaceae) and *Dipterocarpus* (Dipterocarpaceae), and it is characterized by two distinct rainy seasons: the southwest monsoon from May to July and the northeast monsoon from November until January. As these collections do not fit any previously described taxa of *Russula*, we describe two new species by providing detailed descriptions and placing them in a molecular phylogeny. We believe these taxa are likely endemic to the region, as sequestrate Russulaceae often display a limited geographical range, and there is a high degree of faunal and floral endemicity in this area (Lebel 1998). Finally, we discuss the potential evolutionary drivers of this high diversity of sequestrate Russulaceae in these rainforests.

Methods

This study is based on two collections made by Annemieke Verbeken (AV) and Dirk Stubbe (DS) during a field expedition near the Sinharaja Forest Reserve in Sri Lanka in December 2007. The studied material was deposited in the Herbarium Universitatis Gandavensis (GENT).

Morphological description

Microscopical observations were performed from exsiccates using an Olympus CX-31 microscope with a mounted drawing tube or a Nikon Eclipse Ni with a mounted Nikon DS-Fi3 camera. Line drawings of hymenial or pileal structures were made using this drawing tube, and spores were drawn using a Zeiss Axioskop 2 microscope with a mounted drawing tube. Spores were observed after rehydrating lamellae sections in distilled water and staining with Melzer's reagent. Slides of hymenial or pileal structures were prepared by softening the material in 10% KOH for a few seconds, followed by staining in Congo red. Furthermore, thin tissue sections were mounted in Cresyl Blue, sulfovanillin, or HCl and carbolfuchsin to observe reactions with contents or incrustations of elements. Scanning electron microscopy (SEM) images were obtained by mounting a spore print onto an SEM stub, coating the sample with gold, and photographing it with a JEOL JSM–5910 LV SEM (JEOL, Japan) at Meise Botanical Garden.

Spore measurements were taken using Nikon NIS-Elements BR software with an accuracy of 0.01 µm, whereas other structures were measured using an eyepiece with 1 µm accuracy. Statistics of all measurements, as well as the description template, were based on Adamčík et al. (2019). For each collection, 20 to 30 measurements were taken per structure. Average values, including the standard deviation, were calculated and reported as follows: for spore length and width (MIN) [AVG − 2 × SD] AVG [AVG + 2 × SD] (MAX), and for other structures (MIN) [AVG − SD] AVG [AVG + SD] (MAX).

DNA extraction, PCR, and sequencing

DNA extraction and PCR amplification were performed at the Centre for Molecular Evolution & Phylogeny (CEMOFE, Ghent University, Belgium). DNA was extracted from exsiccates using a modified CTAB protocol as described by Nuytinck and Verbeken (2003). From these extracts, two loci were amplified:

the internal transcribed spacer region of the nuclear ribosomal DNA (ITS) using primers ITS1-F and ITS4 (White et al. 1990; Gardes and Bruns 1993) and the nuclear ribosomal large subunit region (LSU) using primers LR0R and LR5 (Vilgalys and Hester 1990; Rehner and Samuels 1994). PCR protocols for each respective locus are described by Huyen et al. (2007).

Sequencing was performed at MACROGEN (Amsterdam, The Netherlands) using an ABI 3730XL. Consensus sequences from ABI files were generated using Geneious v8.1.9 (Kearse et al. 2012).

Phylogenetic analyses

Based on GenBank BLAST results (searches conducted on 28 August 2025), an initial dataset of ITS sequences similar to the newly produced sequences (percentage identity > 85%) was created. By adding sequences from other phylogenies in relevant literature, this dataset was extended. LSU sequences of these specimens were added when available. *Russula nigricans* Fr. 1838 from *Russula* subg. *Compactae* was selected as the outgroup. These sequences and related literature are listed in Table 1. The final dataset comprised sequences from 160 collections, including the outgroup, and was used for further phylogenetic analyses.

ITSx v1.1.3 was used to extract the ITS1, 5.8S, and ITS2 regions from the ITS sequences, and all four loci were aligned separately using the L-INS-I strategy in MAFFT v7 (Bengtsson-Palme et al. 2013; Katoh and Standley 2013) and manually adjusted with PhyDE® v0.9971 (Müller et al. 2010). Alignments were trimmed using Gblocks with the following parameters: default settings, half gaps allowed, and a minimum block length of 2 (Castresana 2000). Phylogenetic analyses were performed separately for each individual locus and for all concatenated loci using Bayesian inference (BI), as implemented in MrBayes v3.2 (Ronquist et al. 2012), and maximum likelihood (ML), as implemented in RAxML 7.0.4 (Stamatakis 2006). Model selection was performed using jModelTest2 (Darriba et al. 2012), and the GTR+I+G model was selected for both loci based on the AICc criterion. The best-fit models for each partition were implemented as partition-specific models within partitioned mixed-model analyses of the combined dataset, and all parameters were unlinked across partitions.

The combined dataset Bayesian analyses were implemented with four independent runs, each with four simultaneous independent chains for 10 million generations, starting from random trees and sampling one tree every 1000th generation. Convergence was assessed based on the standard deviation of split frequencies and effective sample sizes (ESS) of parameters. All trees sampled after convergence, defined by an average standard deviation of split frequencies < 0.01 and confirmed using Tracer v1.4 (Rambaut et al. 2018), were used to reconstruct a 50% majority-rule consensus tree (BC) and to calculate Bayesian posterior probabilities (PP). The PP of each node was estimated based on the frequency with which the node was resolved in the sampled trees using the 50% majority-rule consensus option (Simmons et al. 2004). A probability of 0.95 was considered significant. The two Bayesian runs converged to stable likelihood values after 5,585,000 generations. Therefore, 4,415 stationary trees from each analysis were used to construct the 50% majority-rule consensus tree and to calculate posterior probabilities (PP).

ML searches were conducted with RAxML, involving 1,000 replicates under the GTRGAMMAI model, with all model parameters estimated by the program. In addition, 1,000 bootstrap (ML BS) replicates were run using the same GTRGAMMAI model. Clades with ML BS values of 75% or greater were considered supported by the data. Before combining the data partitions, topological incongruence between the datasets was assessed using 1,000 ML BS replicates under the same models described above for each locus separately. Paired trees were examined for conflicts involving only nodes with ML BS ≥ 75% (Mason-Gamer and Kellogg 1996; Lutzoni et al. 2004; Reeb et al. 2004), and the results were compared with those obtained using the software compat.py (Kauff and Lutzoni 2002). A conflict was assumed to be significant if two different relationships for the same set of taxa, one being monophyletic and the other non-monophyletic, were observed in rival trees.

Table 1. Specimens and GenBank accession numbers of sequences used in the phylogenetic analyses. The species name, specimen voucher, country where the specimen was collected, accession numbers of the ITS and LSU sequences (when available), and the study under which these sequences were published (when available) are provided (/ indicates missing data).

Species	Voucher	Country	ITS	LSU	Reference
Russula aff. *roseostipitata*	JAC16499	New Zealand	OR348188	OR343277	(Buyck et al. 2024)
Russula albocarpa	PDD:69223	New Zealand	OR348212	OR343280	(Buyck et al. 2024)
Russula amarissima	FH 2010 BT42	Germany	MN130064	MN130118	(Adamčík et al. 2019)
Russula amarissima	SAV 1085	Italy	MN130065	MN130119	(Adamčík et al. 2019)
Russula amarissima	SAV F-2412	Slovakia	MN130117	MN130063	(Adamčík et al. 2019)
Russula amethystina	hue215	Germany	AF418640	/	(Eberhardt 2002)
Russula atrovirens	PDD:77744	New Zealand	GU222260	/	/
Russula atroviridis	JAC13171	New Zealand	MW683818	MW683655	/
Russula atroviridis	JAC13218	New Zealand	MW683828	MW683665	/
Russula aurantioflava	LAH 35408	Pakistan	MN130070	MN130121	(Adamčík et al. 2019)
Russula aurantioflava	LAH 35410	Pakistan	MN130069	MN130120	(Adamčík et al. 2019)
Russula aurantiolutea	CMMF024882	Canada	OQ322097	/	/
Russula aurata	2-2210IS77	Europe	AY061659	/	(Miller and Buyck 2002)
Russula aurata	fruitbody137	China	MN704815	MN710556	(Xing et al. 2020)
Russula aurata	fruitbody3	China	MN704814	MN710555	(Xing et al. 2020)
Russula aurata	HKAS 78361	China	KF002751	/	/
Russula aurata	TJ08130	China	PP911722	/	/
Russula aurea	HE2784	China	KC505573	/	/
Russula aurea	HMJAU56941	China	MW517306	/	/
Russula aurea	SAV F-4196	Slovakia	KY582718	/	/
Russula azurea	PC:BB08.668	Italy	JN944002	KU237529	(Schoch et al. 2012)
Russula botrytigustata sp. nov.	AV 07-177	Sri Lanka	PX220002	PX220006	This study
Russula burlinghamiae	PC:BB05.108	USA	MK929285	/	(Wang et al. 2019)
Russula candidissima	JMV 20110906-6a	Spain	MK105636	MK105713	(Vidal et al. 2019)
Russula candidissima	JMV 800664	Spain	MK105634	/	(Vidal et al. 2019)
Russula castanopsidis	XHW3958	China	MN134532	MN134540	(Rossi et al. 2020)
Russula castanopsidis	XHW4334	China	MN134533	MN134541	(Rossi et al. 2020)
Russula cf. *flavisiccans*	PC:BB04.219	USA	EU598156	EU598156	/
Russula cf. *flavisiccans*	PC:BB04.254	USA	EU598162	EU598162	/
Russula cf. *fragilis*	BPL 273	USA	KT933972	KT933833	(Looney et al. 2016)

Species	Voucher	Country	ITS	LSU	Reference
Russula cf. *laccata*	UBC:F30302	Canada	KX812849	KX812870	/
Russula ciceriformis sp. nov.	DS 07-508	Sri Lanka	PX220001	PX220005	This study
Russula claroflava	FH 12-212	Germany	KT933997	KT933858	(Looney et al. 2016)
Russula clelandii	AF95	Australia	DQ328136	/	/
Russula cooperiana	OTA-61381	New Zealand	JX178493	/	/
Russula cooperiana	OTA-75944	New Zealand	PV539428	/	/
Russula corallina	BPL851	USA	KY509449	/	(Looney et al. 2020)
Russula darjeelingensis	CAL 1609	India	MG321326	/	(Paloi et al. 2018)
Russula ellipsospora	OSC 58973	USA	AY239306	/	/
Russula emetica	Prilba	Europe	OL739383	OL739383	(Miyauchi et al. 2020)
Russula flavida	CMMF024703	Canada	OQ322559	/	/
Russula flavida	PC:BB04.218	USA	EU598170	/	/
Russula flavida	PC:BB04.250	USA	EU598171	/	/
Russula flavida	RFRMU085	Thailand	MW468068	/	/
Russula flavida	RMUKH25	Thailand	KX267650	/	/
Russula flavida	RMUKH26	Thailand	KX267651	/	/
Russula flavida	RMURF084	Thailand	MW468067	/	/
Russula flavisiccans	FLAS F-71960	USA	OR664078	/	/
Russula flavisiccans	CMMF002450	Canada	OQ322034	/	/
Russula fragilis	F-3217	Sweden	PQ639013	PQ639013	/
Russula fragilis	FH 12-197	Germany	KT933993	KT933854	(Looney et al. 2016)
Russula griseoviolacea	FUNNZ2017/1911	New Zealand	MW461610	/	/
Russula griseoviolacea	JAC10470	New Zealand	MW683742	MW683614	/
Russula griseoviolacea	JAC11223	New Zealand	MW683776	MW683682	/
Russula griseoviolacea	JAC13273	New Zealand	MW683834	MW683671	/
Russula griseoviolacea	PDD:101446	New Zealand	OR348264	/	(Buyck et al. 2024)
Russula griseoviolacea	PDD:101447	New Zealand	OR348265	OR343264	(Buyck et al. 2024)
Russula hobartiae	JMV800647	Cyprus	MK105651	MK105720	(Vidal et al. 2019)
Russula hobartiae	ML4193GY	Cyprus	MK105648	MK105718	(Vidal et al. 2019)
Russula integra	F-1323	Sweden	PQ653101	PQ653101	/
Russula integra	F-1326	Sweden	PQ653102	PQ653102	/
Russula intermedia	CLC 3784	USA	MT583251	/	(Noffsinger and Cripps 2021)
Russula intermedia	CLC 3822	USA	MT583250	/	(Noffsinger and Cripps 2021)
Russula kalimna	MEL:2238306	Australia	EU019927	/	(Lebel and Tonkin 2007)
Russula laccata	TU<EST>:101871	USA	KX812854	KX812890	/
Russula lepida	69IJ62	Czech Republic	MG687359	/	(Leonhardt et al. 2019)
Russula lepida	HJB9990	Europe	DQ422013	DQ422013	/
Russula lepida	hue208	Germany	AF418641	AF325310	(Eberhardt 2002)
Russula lilacea	PC:BB07.213	Slovakia	JN944005	JN940592	(Schoch et al. 2012)
Russula macrocystidiata	JAC12305	New Zealand	MW683802	MW683640	/
Russula macrocystidiata	JAC12918	New Zealand	MW683808	MW683645	/
Russula macrocystidiata	JAC13271	New Zealand	MW683832	MW683669	/
Russula macrocystidiata	JAC16410	New Zealand	OR348184	OR343274	(Buyck et al. 2024)
Russula mattiroloana	JMV800713	Poland	MK105656	MK105723	(Vidal et al. 2019)
Russula mattiroloana	JMV800644	Greece	MK105653	MK105722	(Vidal et al. 2019)
Russula meridionalis	IC20051417	Spain	MK105664	MK105727	(Vidal et al. 2019)
Russula messapica var. *messapica*	JL201111182	Spain	MK105669	MK105730	(Vidal et al. 2019)
Russula messapica var. *messapicoides*	VK2998	Greece	MK105670	MK105731	(Vidal et al. 2019)

Species	Voucher	Country	ITS	LSU	Reference
Russula miniata	JAC14570	New Zealand	MW683849	MW683682	/
Russula minutula	BPL575	Slovakia	KY509455	/	(Looney et al. 2020)
Russula nigricans	UBC F23780	Canada	KC581314	KC581314	/
Russula nigricans	UE20-09-2004-07	Germany	DQ422010	DQ422010	/
Russula olivascens	F-1159	Sweden	PQ653065	PQ653065	/
Russula olivascens	F-4982	Sweden	PQ652299	PQ652299	/
Russula osphranticarpa	JAC13799	New Zealand	MW683840	MW683677	/
Russula osphranticarpa	JAC16361	New Zealand	OR348178	OR343272	(Buyck et al. 2024)
Russula paludosa	F-1201	Sweden	PQ653402	PQ653402	/
Russula paludosa	MV-1782	Sweden	PQ652460	PQ652460	/
Russula peckii	BPL270	USA	KT933970	KT933830	(Looney et al. 2016)
Russula pelargonia	F-2182	Sweden	PQ652678	PQ652678	/
Russula pelargonia	F-3867	Sweden	PQ639215	PQ639215	/
Russula pilocystidiata	JAC12529	New Zealand	MW683806	MW683644	/
Russula pilocystidiata	JAC12921	New Zealand	MW683810	MW683647	/
Russula puellaris	F-2461	Sweden	PQ652697	PQ652697	/
Russula puellaris	RITF2987	China	PP102004	PP102135.	/
Russula purpureoflava	JET1128	Australia	JX266626	JX266641	(Lebel et al. 2013)
Russula purpureoflava	MEL2101866	Australia	EU019914	/	(Lebel and Tonkin 2007)
Russula purpureoflava	MEL2101866	Australia	EU019914	/	(Lebel and Tonkin 2007)
Russula purpureogracilis	XHW4521	China	MN134534	MN134542	(Rossi et al. 2020)
Russula purpureogracilis	FH 12-055	Thailand	MN130099	/	(Adamčík et al. 2019)
Russula risigallina	F-2744	Sweden	PQ652616	PQ652616	/
Russula risigallina	F-2746	Sweden	PQ652618	PQ652618	/
Russula romellii	F-2268	Sweden	PQ652756	PQ652756	/
Russula romellii	F-408	Sweden	PQ653008	PQ653008	/
Russula romellii	FH 12-177	Germany	KT933987	KT933848	(Looney et al. 2016)
Russula rosacea	RMUKK35	Thailand	KX267624	/	/
Russula rosea	HKAS 78401	China	KF002785	/	(Guo et al. 2014)
Russula rosea	PC:BB07.780	France	JN944003	JN940602	(Schoch et al. 2012)
Russula roseopileata	PDD:107589	New Zealand	OR348312	OR343293	(Buyck et al. 2024)
Russula roseopileata	PL760619	New Caledonia	MZ828064	MZ827900	/
Russula roseostipitata	JAC16311	New Zealand	MW683885	MW683712	/
Russula roseostipitata	PDD:92050	New Zealand	GU222324	/	/
Russula roseostipitata	PDD:88997	New Zealand	GU222285	/	/
Russula rubellipes	BPL240	USA	KT933958	KT933817	(Looney et al. 2016)
Russula rubrolutea	JAC14704	New Zealand	MW683853	MW683683	/
Russula rubrolutea	PDD:83697	New Zealand	OR348222	/	(Buyck et al. 2024)
Russula rubrolutea	Trappe12610	Australia	EU019940	EU019940	(Lebel and Tonkin 2007)
Russula seminuda	H5346	Australia	EU019947	/	(Lebel and Tonkin 2007)
Russula sessilis	H5038	Australia	EU019948	EU019948	(Lebel and Tonkin 2007)
Russula sp.	FH00304560	Pakistan	MN130077	/	(Adamčík et al. 2019)
Russula sp.	FLAS_F_61206	USA	MH211811	/	/
Russula sp.	JAC10922	New Zealand	MW683759	/	/
Russula sp.	JAC15267	New Zealand	OR348175	/	(Buyck et al. 2024)
Russula sp.	JAC15845	New Zealand	MW683861	MW683688	/
Russula sp.	JAC16031	New Zealand	MW683872	MW683699	/
Russula sp.	JAC16032	New Zealand	MW683873	MW683700	/

Species	Voucher	Country	ITS	LSU	Reference
Russula sp.	JAC16034	New Zealand	MW683874	MW683701	/
Russula sp.	MHM215	Mexico	EU569278	/	(Morris et al. 2008)
Russula sp.	PDD:101496	New Zealand	OR348302	/	(Buyck et al. 2024)
Russula sp.	JAC13248	New Zealand	MW683830	MW683666	/
Russula sp.	PDD:89034	New Zealand	GU222292	/	/
Russula sp.	r-04013	USA	JF834347	JF834495	/
Russula longisterigmata	Trappe 26265	Chile	KF819808	/	(Trierveiler-Pereira et al. 2015)
Russula lauradomingueziae	Trappe 26311	Chile	KF819811	/	(Trierveiler-Pereira et al. 2015)
Russula gamundiae	Trappe 26316	Chile	KF819810	/	(Trierveiler-Pereira et al. 2015)
Russula nothofagi	Trappe 26350	Chile	KF819809	/	(Trierveiler-Pereira et al. 2015)
Russula sp. AZ Gymno	JLF11812	USA	OR722664	/	/
Russula sp. ECM	Dipt10-SL2B	Indonesia	LC482572	/	/
Russula sp. ECM	Dipt10-SS10B	Indonesia	LC482582	/	/
Russula sp. ECM	Dipt2-SM10A	Indonesia	LC482610	/	/
Russula sp. ECM	Dipt2-SM5B	Indonesia	LC482550	/	/
Russula sp. VH-2023a	PERTH 07710259	Australia	OR441040	/	(Buyck et al. 2023)
Russula spinispora	PDD:61990	New Zealand	OR348210	/	(Buyck et al. 2024)
Russula subvinosa	JAC13167	New Zealand	MW683814	MW683651	/
Russula subvinosa	JAC13172	New Zealand	MW683819	MW683656	/
Russula tapawera	PDD:83696	New Zealand	OR348221	/	(Buyck et al. 2024)
Russula tapawera	Trappe12611	Australia	EU019942	/	(Lebel and Tonkin 2007)
Russula tapawera	Trappe12607	Australia	EU019935	EU019935	(Lebel and Tonkin 2007)
Russula tawai	JAC16095	New Zealand	MW683878	MW683705	/
Russula tawai	JAC16551	New Zealand	MW683886	MW683713	/
Russula theodoroui	SLM43I84	Australia	DQ403804	/	(Smith et al. 2006)
Russula tricholomopsis	PDD:77749	New Zealand	GU222261	/	/
Russula turci	UBC:F16268	Canada	EF530935	EF530935	/
Russula wielangtae	HO 593331	Australia	MN130115	/	(Adamčík et al. 2019)
Russula wielangtae	HO 593334	Australia	MN130116	/	(Adamčík et al. 2019)
Russula wollumbina	MEL2238232	Australia	EU019921	EU019921	(Lebel and Tonkin 2007)
Russula xantho	iNaturalist 178260830	USA	PQ822136	/	/
Russula xantho	CMMF001718	Canada	OQ322583	/	/
Russula xantho	HRL3396	Canada	OQ322502	/	/
Russula xerophila	iNaturalist 208196167	USA	PQ368443	/	/

Results

Phylogeny

The final combined DNA sequence alignment contains 160 sequences and 1845 characters, including gaps (160 sequences and 929 characters in the ITS partition and 90 sequences and 926 characters in the LSU partition). This alignment contains two new ITS sequences and two new LSU sequences generated in this study. No conflicts involving significantly supported nodes were found between the tree topologies obtained for the individual loci datasets. The consensus trees from the BI and ML analyses are congruent with respect to the terminal clades or supported lineages. The phylogeny shows overall support for groups within the genus *Russula* previously recognized at section or

subsection rank. The nomenclature of these infrageneric groups was assigned based on current taxonomic literature. The annotated consensus tree with support values for the significantly supported branches is displayed in Figs 1, 2.

The concatenated phylogeny places these collections in the crown clade of *Russula* subg. *Russula*. Both collections represent distinct taxa based on their phylogenetic positions, and we describe them here as *R. botrytigustata* sp. nov. and *R. ciceriformis* sp. nov. *R. botrytigustata* forms a well-supported, isolated lineage with a few environmental sequences isolated from *Shorea* spp. root tips from Sumatra, Indonesia (ML BS = 100, PP = 1). It appears to be in a sister relationship to the clade containing the *R. tapawera* and *R. castanopsidis* lineages (ML BS = 100, PP = 1). All of these lineages form a well-supported clade that probably deserves classification at the subsection level. Interestingly, the phylogenetic signal in the ITS sequence of *R. botrytigustata* differs significantly between the ITS1 and ITS2 regions. Whereas ITS1 is highly variable and of limited informative value, ITS2 offers better resolution and places this species in close relationship with this sister clade. The 5.8S region is also unusually divergent from species belonging to closely related lineages but is almost identical to the environmental sequences in this new clade. *Russula ciceriformis* is positioned either within or as a sister to the *R. wielangtae* lineage, but this placement is weakly supported (ML BS = 39, PP = 0.55). Consistent with previous studies,

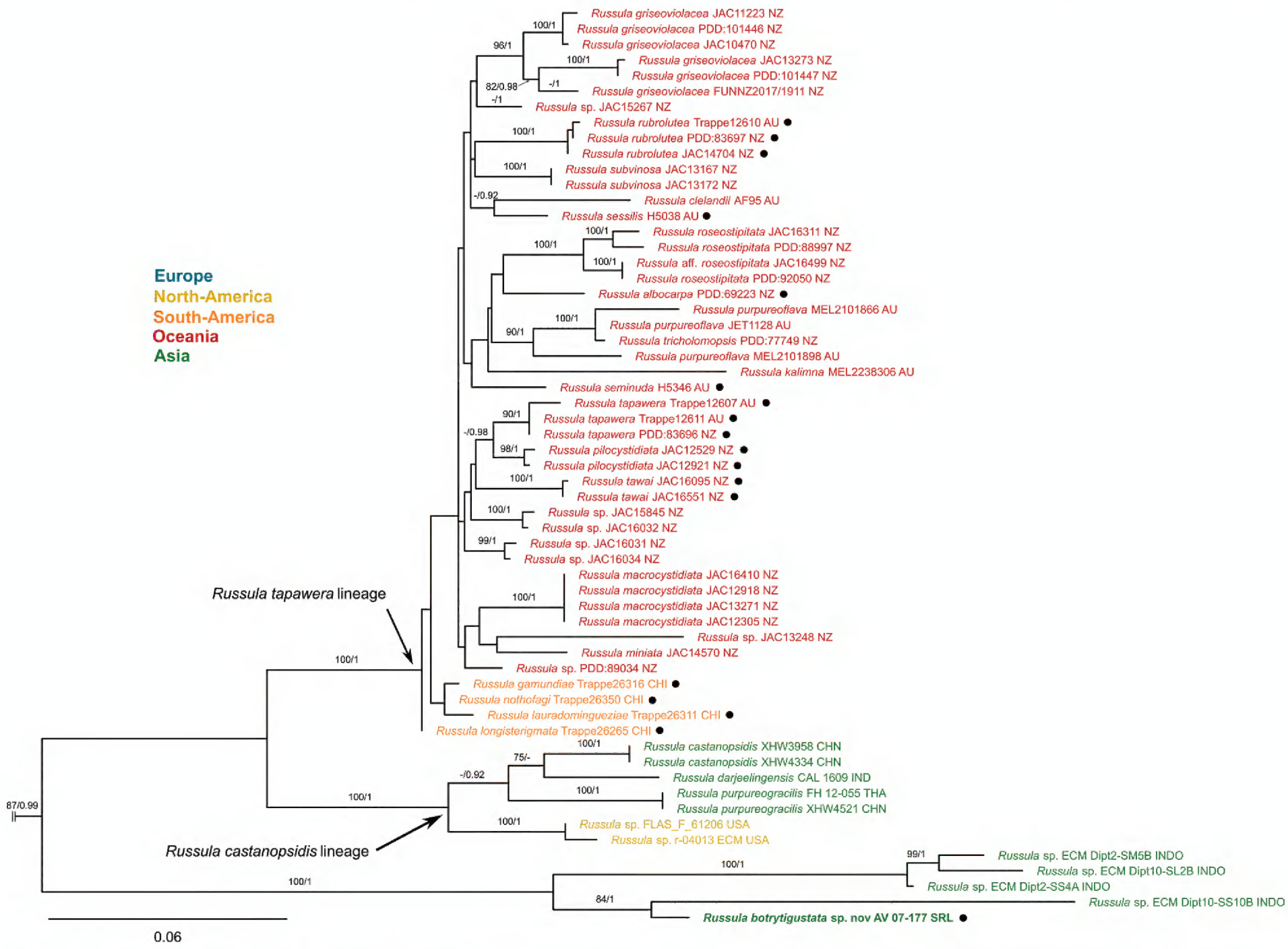

Figure 1. Concatenated ML tree of ITS and LSU sequences of *Russula* subg. *Russula* crown group containing *Russula botrytigustata* sp. nov., continuing in Fig. 2. Bootstrap support values (ML BS) ≥ 75 and posterior probabilities (PP) ≥ 0.90 are reported (ML BS/PP). New taxa are indicated in bold, and sequestrate taxa are indicated with ●.

the *R. aurea* and *R. wielangtae* lineages form a strongly supported clade and are considered to constitute *Russula* subsect. *Auratinae* Bon based on their microscopical similarities, while other studies suggest synonymizing *R.* subsect. *Auratinae* with the *R. aurea* lineage alone. It is difficult to resolve the exact positions of both species within the subgenus using the ITS and LSU loci, probably due to the lack of closely related species.

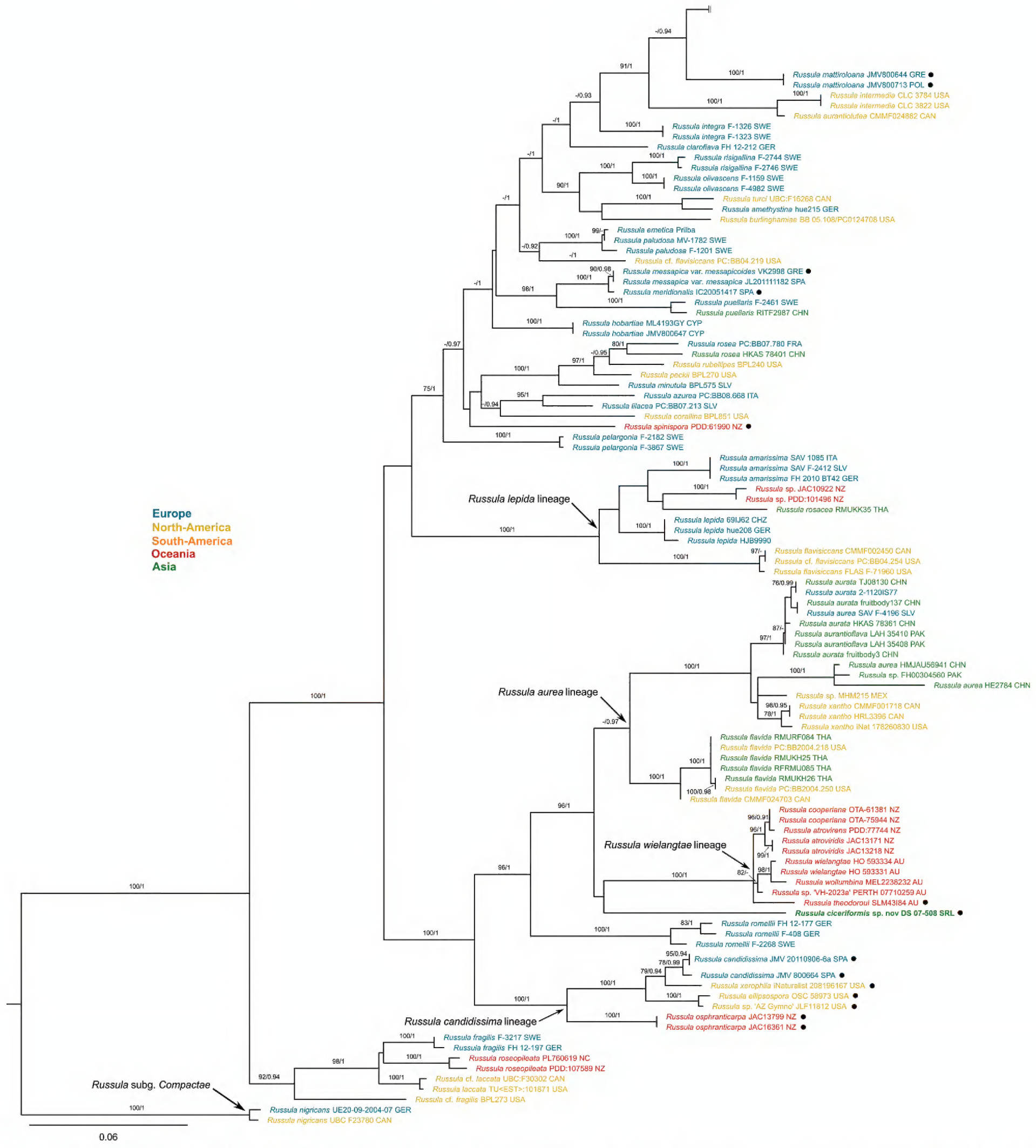
Europe
North-America
South-America
Oceania
Asia
Russula lepida lineage
Russula aurea lineage
Russula wielangtae lineage
Russula candidissima lineage
Russula subg. Compactae
Russula mattiroloana JMV800644 GRE
Russula mattiroloana JMV800713 POL
Russula intermedia CLC 3784 USA
Russula intermedia CLC 3822 USA
Russula aurantiolutea CMMF024882 CAN
Russula integra F-1326 SWE
Russula integra F-1323 SWE
Russula clarofiava FH 12-212 GER
Russula risigallina F-2744 SWE
Russula risigallina F-2746 SWE
Russula olivascens F-1159 SWE
Russula olivascens F-4982 SWE
Russula turci UBC:F16268 CAN
Russula amethystina hue215 GER
Russula burlinghamiae BB 05.108/PC0124708 USA
Russula emetica Prilba
Russula paludosa MV-1782 SWE
Russula paludosa F-1201 SWE
Russula cf. flavisiccans PC:BB04.219 USA
Russula messapica var. messapicoides VK2998 GRE
Russula messapica var. messapica JL201111182 SPA
Russula meridionalis IC20051417 SPA
Russula puellaris F-2461 SWE
Russula puellaris RITF2987 CHN
Russula hobartiae ML4193GY CYP
Russula hobartiae JMV800647 CYP
Russula rosea PC:BB07.780 FRA
Russula rosea HKAS 78401 CHN
Russula rubellipes BPL240 USA
Russula peckii BPL270 USA
Russula minutula BPL575 SLV
Russula azurea PC:BB08.668 ITA
Russula lilacea PC:BB07.213 SLV
Russula corallina BPL851 USA
Russula spinispora PDD:61990 NZ
Russula pelargonia F-2182 SWE
Russula pelargonia F-3867 SWE
Russula amarissima SAV 1085 ITA
Russula amarissima SAV F-2412 SLV
Russula amarissima FH 2010 BT42 GER
Russula sp. JAC10922 NZ
Russula sp. PDD:101496 NZ
Russula rosacea RMUKK35 THA
Russula lepida 69IJ62 CHZ
Russula lepida hue208 GER
Russula lepida HJB9990
Russula flavisiccans CMMF002450 CAN
Russula cf. flavisiccans PC:BB04.254 USA
Russula flavisiccans FLAS F-71960 USA
Russula aurata TJ08130 CHN
Russula aurata 2-1120IS77
Russula aurata fruitbody137 CHN
Russula aurea SAV F-4196 SLV
Russula aurata HKAS 78361 CHN
Russula aurantioflava LAH 35410 PAK
Russula aurantioflava LAH 35408 PAK
Russula aurata fruitbody3 CHN
Russula aurea HMJAU58941 CHN
Russula sp. FH00304560 PAK
Russula aurea HE2784 CHN
Russula sp. MHM215 MEX
Russula xantho CMMF001718 CAN
Russula xantho HRL3396 CAN
Russula xantho iNat 178260830 USA
Russula flavida RMURF084 THA
Russula flavida PC:BB2004.218 USA
Russula flavida RMUKH25 THA
Russula flavida RFRMU085 THA
Russula flavida RMUKH26 THA
Russula flavida PC:BB2004.250 USA
Russula flavida CMMF024703 CAN
Russula cooperiana OTA-61381 NZ
Russula cooperiana OTA-75944 NZ
Russula atrovirens PDD:77744 NZ
Russula atroviridis JAC13171 NZ
Russula atroviridis JAC13218 NZ
Russula wielangtae HO 593334 AU
Russula wielangtae HO 593331 AU
Russula wollumbina MEL2238232 AU
Russula sp. 'VH-2023a' PERTH 07710259 AU
Russula theodoroui SLM43I84 AU
Russula ciceriformis sp. nov DS 07-508 SRL
Russula romellii FH 12-177 GER
Russula romellii F-408 GER
Russula romellii F-2268 SWE
Russula candidissima JMV 20110906-6a SPA
Russula candidissima JMV 800664 SPA
Russula xerophila iNaturalist 208196167 USA
Russula ellipsospora OSC 58973 USA
Russula sp. 'AZ Gymno' JLF11812 USA
Russula osphranticarpa JAC13799 NZ
Russula osphranticarpa JAC16361 NZ
Russula fragilis F-3217 SWE
Russula fragilis FH 12-197 GER
Russula roseopileata PL760619 NC
Russula roseopileata PDD:107589 NZ
Russula cf. laccata UBC:F30302 CAN
Russula laccata TU<EST>:101871 USA
Russula cf. fragilis BPL273 USA
Russula nigricans UE20-09-2004-07 GER
Russula nigricans UBC F23780 CAN
0.06

Figure 2. Concatenated ML tree of ITS and LSU sequences of *Russula* subg. *Russula* crown group containing *Russula ciceriformis* sp. nov., rooted with *Russula* subg. *Compactae* as the outgroup. Bootstrap support values (ML BS) ≥ 75 and posterior probabilities (PP) ≥ 0.90 are reported (ML BS/PP). New taxa are indicated in bold, and sequestrate taxa are indicated with ●.

Taxonomy

***Russula botrytigustata* Tondeleir & Verbeken, sp. nov.**
MycoBank No: MB861796
Figs 3, 5

Diagnosis. Sequestrate with strongly reduced columella, pileus yellowish white; spores with an almost complete reticulum; hymenial cystidia lageniform, mucronate; pileocystidia cylindrical to subclavate, long and slender.

Holotype. Sri Lanka • A. Verbeken 07-177 (GENT); near Sinharaja Forest; 16 Dec. 2007.

Etymology. *botrytigustata* (Lat. adj.): from *botrytis*, the varietal epithet of *Brassica oleracea* var. *botrytis* (cauliflower), and *gustata* ("tasted"), referring to the distinctive cauliflower-like taste of the basidiomata.

Description. Basidiomata sequestrate, epigeous, subglobose, somewhat flattened, 15 mm in diameter. **Columella** strongly reduced, greyish hyaline. **Pileipellis** yellowish white (4A2), smooth, with gelatinous veins in transparency. **Hymenophore** greyish yellow (4B3-4), with labyrinthoid loculi that are longer towards the stipe. **Context** without odor; taste distinctly like raw cauliflower; chemical reactions not observed. **Spore print** not observed.

Basidiospores 5.6–7.4–9.2(–9.4) × 5.0–6.3–7.5(–7.8) µm, some spores substantially larger, likely originating from 2-spored basidia, then 10.5–12.2 µm, broadly ellipsoid, Q = (1.05–)1.11–1.18–1.25(–1.28); thick-walled; ornamentation of moderately distant, 0.2–0.4 µm high amyloid warts [4–5 in a 3 µm diam. circle], (sub)reticulated and without isolated elements, fused by long ridges [4–5(–8) in a 3 µm diam. circle]; suprahilar spot amyloid with similar but lower ornamentation; suprahilar appendix 0.5 to 1.5 µm long, without sterigmatal appendix. **Basidia** clavate, 2-, 3-, or 4-spored, (24.2–)25.8–31.3–36.8(–43.5) × (6.6–)7.9–9.3–10.6(–12.0) µm; sterigmata 4–7 µm long and curved or rarely straight, thin-walled, hyaline. Basidioles narrowly clavate to clavate, 7–9 µm wide, hyaline. **Hymenial cystidia** widely dispersed, 40–50 per mm^2, lageniform, mucronate, (33.3–)42.6–51.9–61.2(–67.7) × (7.8–)8.3–9.9–11.4(–13.8) µm, extending up to 20 µm from the hymenium, with a (5–)15–30 µm long, tortuous appendix that is rarely branched; contents heteromorphous, coarsely crystalline or hyaline, without reaction in sulfovanillin, turning yellowish in Melzer. **Subhymenium** consisting of 1–2 tiers of cells, parenchymatous to ramose, 9–13 × 13–19 µm. **Hymenophoral trama** consisting of sphaerocytes of diameter 12–24 µm, with few cystidioid hyphae. **Pileipellis** orthochromatic in Cresyl Blue, 140 µm thick, gradually passing into the underlying trama. Suprapellis 15–25 µm thick, ungelatinized, consisting of compact flexuous hyphae with ascending to erect hyphal terminations, gradually passing into thin subpellis, consisting of loose 3–4 µm thick hyphae and 4–5 µm thick cystidioid hyphae. **Hyphal terminations** near the pileus center dispersed and inconspicuous, narrow and thin-walled, occasionally branched, cylindrical, nodulose and flexuose, rarely apically obtuse, sometimes encrusted; terminal cells (21–)27.6–36.5–45.4(–49) × (2.5–)2.7–3.0–3.2(–3.5) µm. Subterminal cells more or less equal in size, rarely branched. Hyphal terminations near the pileus margin similar in shape but slightly shorter; (13.7–)26.3–34.1–42.0(–49) × (2.1–)2.6–3.0–3.5(–3.8) µm. **Pileocystidia** near the pileus center abundant,

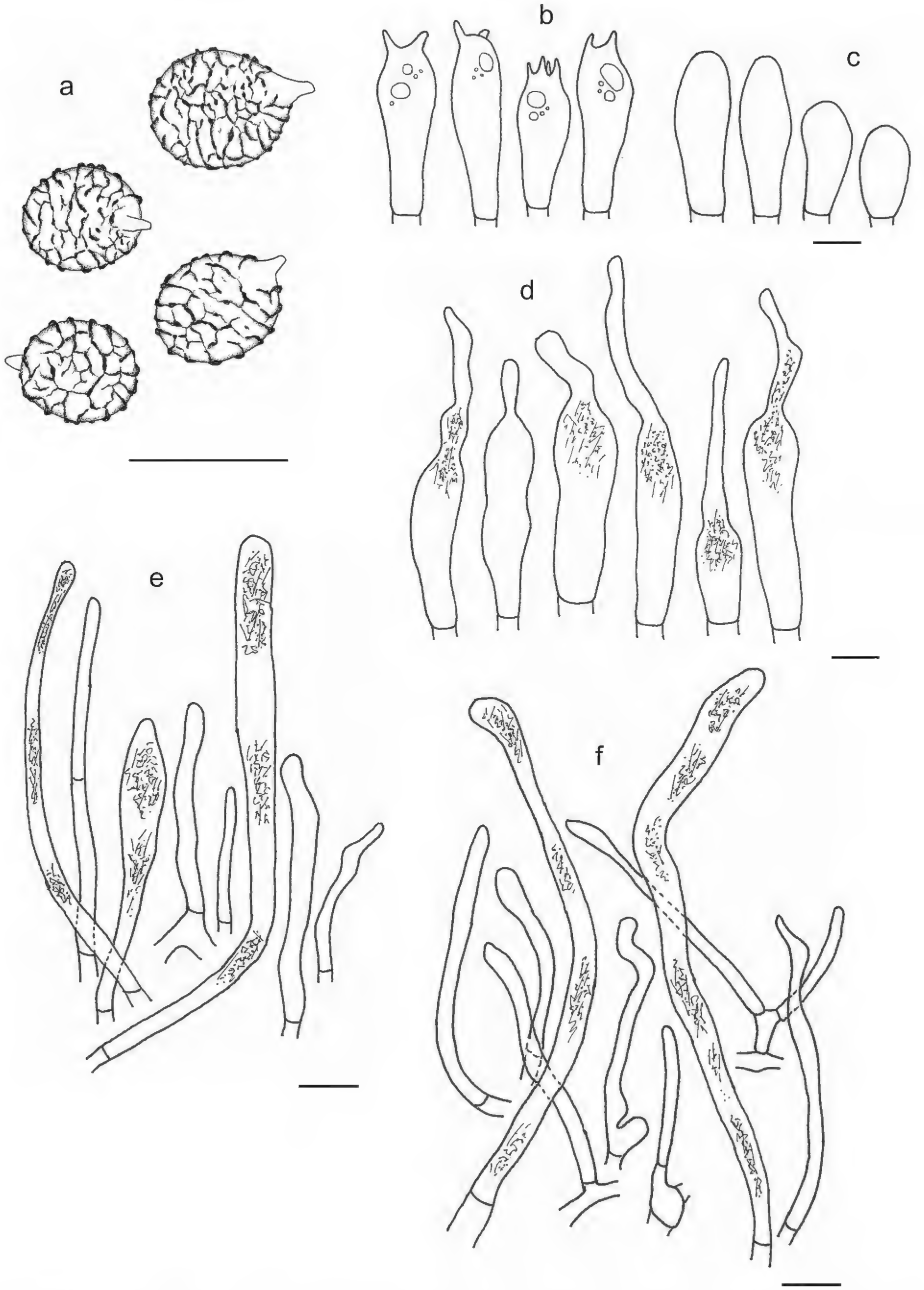

Figure 3. Morphological drawings of *Russula botrytigustata* sp. nov. (Verbeken 07-177). **a.** Spores; **b.** Basidia; **c.** Basidioles; **d.** Hymenial cystidia; **e.** Hyphal terminations and pileocystidia near the pileus margin; **f.** Hyphal terminations and pileocystidia near the pileus center. Contents as observed in Congo red. Scale bars: 10 µm.

1-celled, (42.0–)54.6–106.5–158.4(–270.0) × (4.0–)4.9–6.1–7.3(–9.0) µm, cylindrical to subclavate, rarely obtuse, extending deep into the subpellis, thin-walled, with heteromorphous, finely to coarsely crystalline contents that are sometimes banded. No reaction in sulfovanillin. Pileocystidia near the pileus margin similar in size, abundance, and shape; 45.0–109.2–173.4(–298.0) × (4.5–)5.0–6.2–7.4(–8.0) µm. **Cystidioid hyphae** abundant in subpellis, scarce in hymenophoral trama. **Oleiferous hyphae** absent.

Habitat. Primary rainforest with *Shorea* spp.

Notes. *Russula botrytigustata* shares barely any morphological characteristics with representatives of the *R. castanopsidis*/*R. tapawera* lineages. Some representatives, such as *R. tapawera* (T. Lebel) T. Lebel, exhibit a similar configuration of the pileipellis, but this feature is highly variable within this lineage (McNabb 1973; Lebel 2002; Adamčík et al. 2019). Moreover, all species in the *R. castanopsidis* lineage exhibit isolated ornamentation with only a few line connections, in contrast to the (sub)reticulated ornamentation of *R. botrytigustata*. The presence of mucronate hymenial cystidia appears to be the only shared characteristic. For this reason, and given its phylogenetically distinct position, we assume that *R. botrytigustata* represents the first described species from a thus far unrecognized lineage.

This species shows some similarities with the Australian sequestrate species *R. parvisaxoides* (T. Lebel) T. Lebel by sharing mucronate hymenial cystidia and lacking a prominent stipe or columella. However, the spores of this species are much larger (8.5–11 × 7–10 µm), with less reticulate ornamentation, and pileocystidia are absent. As no phylogenetic placement of this species has been provided in earlier studies, we performed a BLAST search of the ITS sequence of the type material, which suggests placement in *R.* subsect. *Cyanoxanthinae* Singer. No other sequestrate species from Australasia shows similar spore ornamentation.

Russula ciceriformis **Tondeleir & Stubbe, sp. nov.**

MycoBank No: MB861795

Figs 4, 5

Diagnosis. Sequestrate, lacking a columella, with an ochraceus to yellowish-buff pileus; spores echinulate; hymenial cystidia and pileocystidia absent.

Holotype. Sri Lanka • D. Stubbe 07-508 (GENT); near Sinharaja Forest, trail along the Pitakele River; 17 Dec. 2007.

Etymology. *ciceriformis* (Lat. adj.): from *cicer*, the genus epithet of the chickpea (*Cicer arietinum*), and *formis* ("shaped"), referring to the shape and color of the species, which is reminiscent of a chickpea.

Description. Basidiomata sequestrate, epigeous, subglobose, 11–15 mm in diameter. **Columella** absent. **Pileipellis** pale ochraceus to yellowish-buff (5B5), slightly felty, not translucent but with a few pleats. **Hymenophore** pale yellow (4A3), faintly darkening when dried. Finely labyrinthine loculoid. **Context** with a sweetish, agreeable odor and neutral taste. **Spore print** not observed.

Basidiospores (6.5–)6.6–7.8–9.0 × 6.2–6.9–7.7 µm, subglobose to broadly ellipsoid, Q (1.01–)1.06–1.13–1.19(–1.26), echinulate, with isolated high amyloid spines that are 1.3–2.0 µm high, sometimes with interspersed

lower spines, moderately distant [on average 4–6 spines in a 3 µm diam. circle, on average 0–1 fusions in a 3 µm diam. circle]. Strongly thick-walled. With long hilar appendix up to 2.5 µm long, sometimes with sterigmatal appendix still attached. Suprahilar spot inamyloid but ornamented with slightly lower spines. **Basidia** subclavate to clavate, 2- or 3-spored, rarely 4-spored, hyaline or sometimes with a few oil droplets, (19.5–)26.7–33.6–40.4(–47.5) × (7.2–)8.2–9.1–10.0 µm, sterigmata 2–4 µm long, curved. Basidioles narrowly clavate to clavate, 8–9 µm wide, hyaline. **Hymenial cystidia** absent. **Subhymenium** consisting of 1–3 tiers of cells, pseudoparenchymatous to ramose, 8–10 × 7–9 µm. **Hymenophoral trama** consisting of 4–7 µm-wide long- to short-celled hyphae, lacking cystidioid or oliferous hyphae. **Pileipellis** orthochromatic in Cresyl Blue, 170–230 µm thick, sharply delimited from underlying trama. Suprapellis ungelatinized, 40–50 µm thick, constisting of loosely arranged, erect, 5–7 µm thick hyphae, gradually passing into pallisade subpellis, consisting of globose, 15–20 µm wide cells with a yellow intracellular pigment, as observed in Congo red. **Hyphal terminations** near the pileus center dispersed, variable in number of cells and size; 1–4–8 celled, sometimes slightly tapering or inflated towards the top, unconstricted to slightly constricted at the septa, unbranched, seemingly consisting of two types: septate, cystidia-like, often encrusted with ochraceus pigmentation, or aseptate, attenuated; terminal cells (12.0–)15.1–22.5–29.9(–44.0) × (4.0–)4.6–5.6–6.6(–8.0) µm, variable in shape; either cylindrical to fusiform or attenuated, rarely obtuse, subterminal cells often equally long and wide. Without defined contents, optically empty but with yellow intracellular pigment as observed in Congo red; no reaction in sulfovanillin. Hyphal terminations near the pileus margin very abundant, similar in shape and length but thinner on average and less often with encrustation. Terminal cells (9.0–)9.4–22.9–36.4(–73.0) × (2.5–)3.4–4.5–5.6(–7.0) µm. **Pileocystidia** absent. **Cystidioid and oleiferous hyphae** in the subpellis and context absent.

Habitat. Primary rainforest with *Shorea trapezifolia* and *Dipterocarpus hispidus*.

Notes. *Russula ciceriformis* sp. nov. is the second sequestrate species described from the *R. aurea* or *R. wielangtae* lineages. The only other species is *R. theodoroui* T. Lebel (T. Lebel) from Australia, which is placed in the *R. aurea* lineage (Fig. 1) (Lebel 2003). It shares many morphological characteristics with several species from these lineages. Species belonging to this clade lack distinct pileocystidia but may instead present two types of hyphal terminations: septate, cystidia-like ones and thinner, aseptate, attenuated ones, similar to those observed in *R. ciceriformis* (McNabb 1973; Lebel 2003; Adamčík et al. 2019; Ghosh et al. 2023). *R. flavida* Frost and *R. pseudoflavida* A. Ghosh, Hembrom, I. Bera & Buyck, part of the *R. aurea* lineage, also contain golden encrustations on some elements of the pileipellis that may cause hyphal terminations to resemble primordial hyphae (Adamčík et al. 2018; Ghosh et al. 2023). Although Ghosh et al. (2023) reported an uncertain reaction with Cresyl Blue, we did not observe any reaction and determined that primordial hyphae are absent in *R. ciceriformis*. The isolated spore ornamentation of this taxon is unusual compared with other representatives of the *R. wielangtae* lineage, whose ornamentation is always connected by lines or ridges. Still, given the distinct micromorphological synapomorphy in the pileipellis of *R. ciceriformis*

and other members of the *R. wielangtae* lineage, we believe that it should be included in this lineage. Likely, more undescribed Southeast Asian species belonging to this lineage exist, which would elucidate the relationships among *R. ciceriformis* and related species.

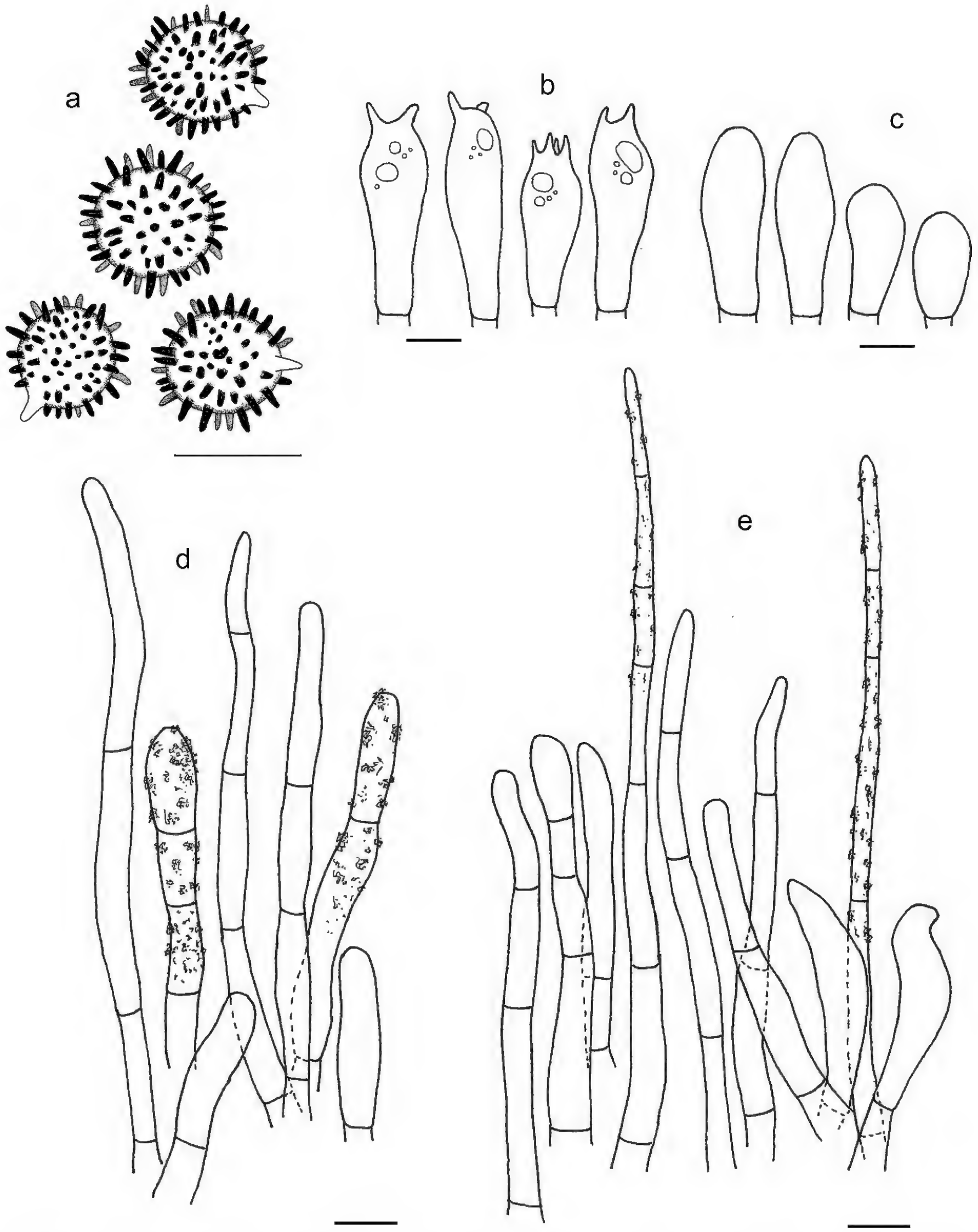

Figure 4. Morphological drawings of pileal and hymenial elements of *Russula ciceriformis* sp. nov. (DS 07-508). **a.** Spores; **b.** Basidia; **c.** Basidioles; **d.** Hyphal terminations of the pileus center; **e.** Hyphal terminations of the pileus margin. Scale bars: 10 µm.

This species shows some morphological similarities with other sequestrate *Russula* species from Australasia. Morphologically, it appears similar to *R. spinispora* T. Lebel, which also has spores with isolated spines, lacks hymenial cystidia, and displays a similar pileus morphology (Lebel 2002, 2017). The phylogeny, however, places this species within the *R. lilacea* lineage (Fig. 2) (Adamčík et al. 2019). Moreover, the spores of *R. spinispora* are larger (8–10.9 × 8–10.4 µm) and more globose (Qavg 1.02–1.05), and this species displays a dark brown hymenium at maturity, both characteristics absent in *R. ciceriformis*. Similarly, *R. leucocarpa* T. Lebel (T. Lebel) displays spores of similar size and shape with isolated spines and lacks hymenial cystidia; however, it also lacks prominent pileocystidia (Lebel 2002, 2017). *R. brevipileocystidiata* X.Y. Sang & L. Fan, *R. subterranea* L. Fan & H.Y. Fu, *R. lithocarpi* W.N. Chou, and *R. megapseudocystidiata* X.Y. Sang & L. Fan, all described from China, show similar spore ornamentation but possess hymenial cystidia (Sang et al. 2016; Fu et al. 2025). *R. absphaerocellaris* X.Y. Sang & L. Fan, also described from China, likewise lacks hymenial cystidia and displays similar spore ornamentation (Sang et al. 2016). However, its spores are much larger (10–15 × 10–15 µm), and this species has a dark brown hymenium. Moreover, all these Chinese species are placed in different subsections based on molecular data.

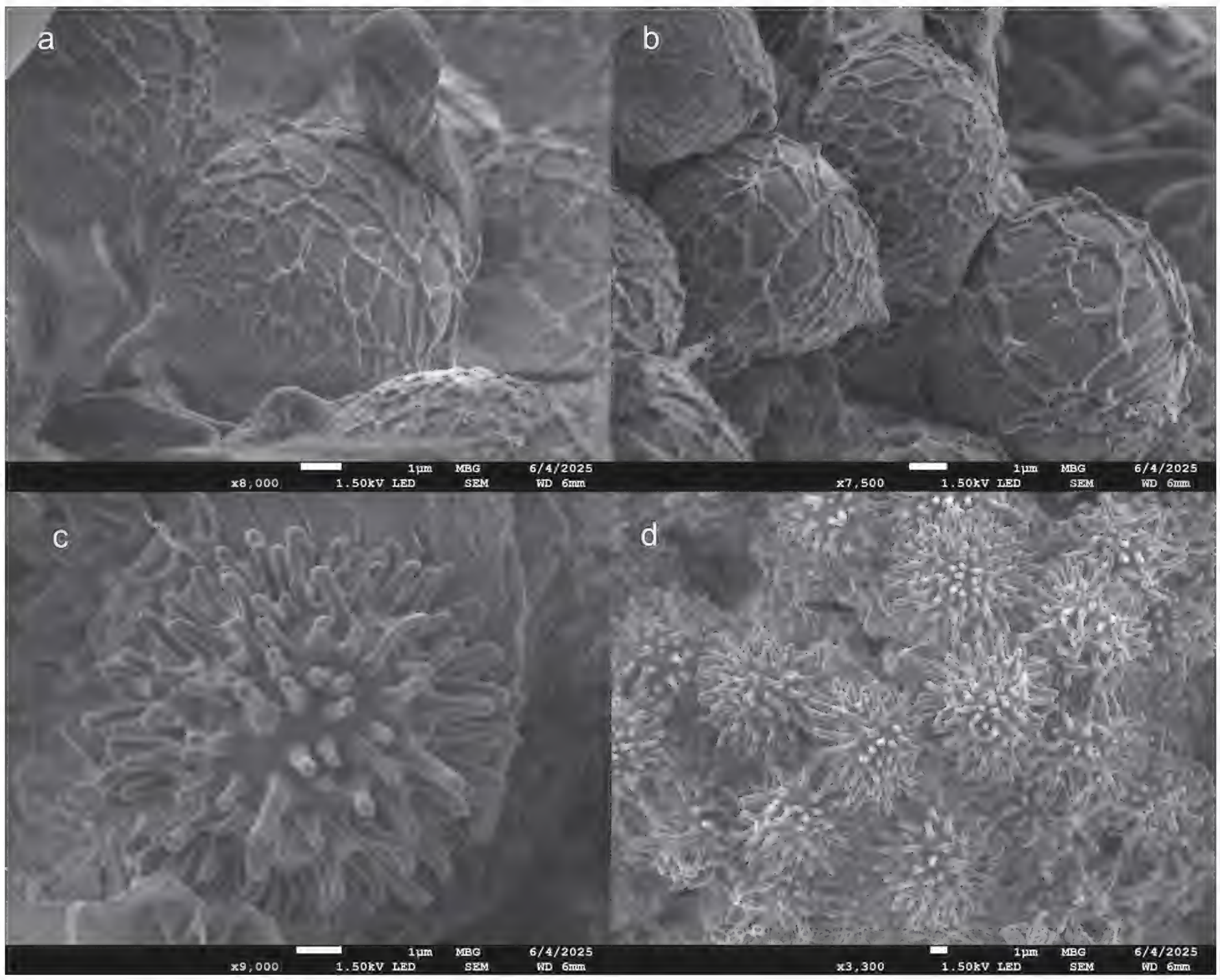
a
b
x8,000 1µm MBG 6/4/2025 1.50kV LED SEM WD 6mm
x7,500 1µm MBG 6/4/2025 1.50kV LED SEM WD 6mm
c
d
x9,000 1µm MBG 6/4/2025 1.50kV LED SEM WD 6mm
x3,300 1µm MBG 6/4/2025 1.50kV LED SEM WD 6mm

Figure 5. SEM images of spores of: **a–b.** *Russula botrytigustata* (AV 07-177); **c–d.** *Russula ciceriformis* (DS 07-508).

Key to sequestrate Russulaceae from South and Southeast Asia

1 Basidiomata exude latex when cut .....(***Lactarius***) **(Verbeken et al. 2014)**
– Basidiomata not exuding latex **2**
2 Hymenial cystidia lacking **3**
– Hymenial cystidia present **4**
3 Basidia 1-spored.....***R. absphaerocellaris*** **X. Y. Sang & L. Fan**
– Basidia 2–4-spored .....***R. ciceriformis*** **Tondeleir & Stubbe**
4 Spores with subreticulated, low ornamentation.....
.....***R. botrytigustata*** **Tondeleir & Verbeken**
– Spores with high ornamentation consisting of isolated or reticulated spines.....**5**
5 Hymenium cream .....***R. lithocarpi*** **W. N. Chou**
– Hymenium pale to dark brown **6**
6 Hymenial cystidia large (up to 90 µm long) and clavate, basidia 2-spored.
.....***R. megapseudocystidiata*** **X. Y. Sang & L. Fan**
– Hymenial cystidia shorter (up to 60 µm), basidia 2-, 3-, or 4-spored **7**
7 Without pileocystidia.....***R. subterranea*** **L. Fan & H. Y. Fu**
– With pileocystidia .....***R. brevipileocystidiata*** **X. Y. Sang & L. Fan**

Discussion

Including these newly described species, the total number of sequestrate Russulaceae known from the Sinharaja Forest Reserve now amounts to seven. Interestingly, each of these species' phenotypes results from independent evolutionary transitions to the sequestrate morphology (Figs 1, 2) (Verbeken et al. 2014). Moreover, during the 2007 expedition to Sri Lanka, several other truffle-like, undescribed species of Hydnangiaceae and Hysterangiaceae were collected. The selective pressures driving the high diversity of sequestrate taxa in some habitats have been the subject of much speculation (Thiers 1984; Bougher and Lebel 2001; Albee-Scott 2007; Gube and Dörfelt 2011; Kuhar et al. 2023). The fact that many Australian lineages of *Russula* or *Lactarius*, such as the *R. tapawera* lineage (Fig. 1), show a high rate of evolutionary shifts to the sequestrate morphology, and their close evolutionary relationship to the sequestrate *Russula* species from Patagonia (*R. tapawera* lineage), might suggest that historical biogeographical and climatic conditions have enabled this evolution (Trierveiler-Pereira et al. 2015; Sheedy et al. 2016). Alternatively, many sequestrate species today occur in arid habitats, where this morphology is hypothesized to be an adaptation that protects fruiting bodies from desiccation. However, a different selective pressure is likely at play in these humid rainforests. As discussed by Verbeken et al. (2014), sequestrate morphology could act as protection against excessive moisture during the monsoons, which were ongoing when these newly described species were collected, as this can lead to rapid rotting and damage to fruiting bodies in tropical environments (De Crop et al. 2018). Indeed, studies have shown that Russulaceae in wet tropical habitats often become ephemeral and produce small fruiting bodies, such as the sequestrate taxa described here, which is hypothesized to be a protection against such conditions (Buyck and Buyck 1990; Piepenbring et al. 2015; Miller et al. 2024; Manz et al. 2025).

The low amount of wind action near the ground in these dense rainforests may also decrease the effectiveness of long-range spore dispersal, which could provide a competitive advantage for zoochoric dispersal strategies (Claridge and Trappe 2005). Moreover, the production of spores of ectomycorrhizal sequestrate fungi near the root zone has been suggested to be evolutionarily advantageous, and this appears to be supported by the observation that the evolutionary shift to a truffle-like morphology often co-occurs with the adoption of an ectomycorrhizal (ECM) lifestyle (Miller et al. 1994; Lebel et al. 2015; Bonito et al. 2025). In many cases, mammals, birds, and insects have been shown to be important spore dispersers of truffle-like fungi (Lamont et al. 1985; Bougher and Lebel 2001; Koch and Aime 2018; Nuske et al. 2018; Caiafa et al. 2021; Brunton-Martin et al. 2024). The high abundance of endemic small mammals in Sri Lankan rainforests may support the evolution and persistence of these species (Wijesinghe and Brooke 2005; Lim 2015). The thick-walled, strongly ornamented spores, often echinulate, of many sequestrate taxa, such as *R. ciceriformis*, possibly reflect an adaptation to animal-mediated dispersal (Fogel and Trappe 1978; Thiers 1984; Truong et al. 2017). Likewise, species of the strongly diversified sequestrate *R. candidissima* or *R. tapawera* lineages (Figs 1, 2) typically show a strongly developed reticulate to spiny spore ornamentation (Singer and Smith 1960; Smith et al. 2006; Vidal et al. 2019). Although also observed in some agaricoid taxa, many sequestrate species display a morphological reduction of sterile elements such as hymenial cystidia, as exemplified in *R. ciceriformis* and *R. xerophila* (M.E. Sm. & Trappe) Trappe & T.F. Elliott, as well as several species of sequestrate *Lactarius* such as *L. echinellus* and *L. megalopterus*. This reduction may lower the energetic costs of fruiting body formation under stressful conditions (Claridge and Trappe 2005; Beenken et al. 2016) or may promote endozoochoric dispersal by reducing the acrid compounds that are present in these cystidia.

Fungal diversity in Southeast Asia remains severely understudied despite recent efforts, even in major lineages such as Russulaceae (Verbeken et al. 2014; Wisitrassameewong et al. 2014b, 2014a, 2015; Hyde et al. 2018; Corrales et al. 2022). This is exemplified by the difficulty in phylogenetically positioning these newly described species, which are likely part of predominantly Southeast Asian lineages. Moreover, the Sinharaja Forest Reserve is likely not the only habitat that harbors such a high diversity of sequestrate species. For example, the environmental sequences from Sumatra, sister to *R. botrytigustata*, may represent another hotspot of sequestrate *Russula* species. Improving our knowledge of the diversity and geographical distribution of these taxa would help us understand how these Australasian lineages evolved. In turn, this is of utmost importance for elucidating the frequent emergence of the sequestrate morphology in mushroom-forming fungi, a striking example of convergent evolution.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Use of AI

The authors declare that no AI was used in the production of this study.

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Author contributions

Conceptualization: LT, AV. Data curation: LT. Formal analysis: LT, MA. Funding acquisition: LT, AV. Methodology: LT, DS, AV, MA. Project administration: AV. Supervision: AV. Visualization: LT. Writing—original draft: LT. Writing—review and editing: LT, AV, MA, DS.

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Data availability

All newly generated ITS and LSU sequences have been uploaded to GenBank under accession numbers PX220001, PX220002, PX220005, and PX220006.

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